

# The difference of Natural Killer cell activities of the people live in the aircraft noise area of Adi Soemarmo Airport Boyolali, Surakarta, Indonesia

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## ABSTRACT

Exposure to noises contributes to a health risk. There is sufficient scientific evidence that noise exposure could induce hearing impairment, hypertension and ischemic heart disease, annoyance, sleep disturbances, and decreased school performance. Other evidence as changes in the immune system and birth defects is limited. The aims of this study is to find out the difference of Natural Killer (NK) cell activities of the people live in the aircraft noise area of Adi Sumarmo Airport, Boyolali, Surakarta, Indonesia. The research design was an analytical with a cross sectional approach, taking location at the Dibal and Gagak Sipat Villages, Ngemplak Sub district, Boyolali District. The study was conducted from June 2008 to June 2009. The number of subjects involved was 39. They were divided into 3 groups. Group 1 was exposed to 52.17 dB of noise level (13 respondents). Group 2 was exposed to 71.79 dB of noise level (13 respondents), and Group 3 was exposed to 92.29 dB of noise level (13 respondents). The cytotoxicities of NK cells was measured by flowcytometric and using a non-radioactive method. The samples were taken using a simple random sampling. The data were analyzed by Anova followed by Post Hoc Test using LSD test completed with Homogenous Subsets. The results showed that the activity of NK cells of Group 1, 2 and 3 were  $12.50 \pm 3.25\%$ ,  $17.20 \pm 3.06\%$ , and  $22.33 \pm 6.30\%$ , respectively. The Anova test showed that there was a significant difference of NK cell activities, in the groups of respondents ( $p = 0.000$ ). In conclusion, there was a significant difference of the NK cell activities of the people live in the aircraft noise area of Adi Sumarmo Airport, Boyolali, Surakarta, Indonesia.

**Key words** : noise-immune system-flowcytometric-blood mononuclear cells-health risk

## INTRODUCTION

In the industrial countries, noise is one of the foremost problems of work health. According to World Health Organization, nearly 14% of the whole workers are exposed to noise of more than 90 dB at their workplaces. Meanwhile, in America more than 20 million people are exposed to noise of more than 85 dB. The research conducted around Heathrow Airport in London showed that the children who were chronically exposed to high levels of aircraft noise had raised annoyance, and they

responded it as a stressor. As a result it had impacts on their cognitive ability as well as on their health condition.<sup>1</sup>

Around the International Airport of Adi Sumarmo, Boyolali, Surakarta, Indonesia with the distance of less than 500 meters from the runway, the noise intensity of the high point activity ranges from 74.42 dB to 95.67. dB, which was measured with Weighted Equivalent Continuous Perceived Noise Level (WECPNL). It was reported that 58.1% of the inhabitants in the region experienced neurological hearing loss, and 65% suffered from

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sleep disturbance.<sup>2</sup> At the same region, the author claimed that the inhabitants exposed to the aircraft noise with the high intensity (their house was less than 500 meters from the runway) had different number of lymphocytes compared to those exposed to the aircraft noise with the low intensity (their house was more than 1.000 meters from the runway).

According to The Decree of Director General of Air Transportation Number: SKEP/109/VI/2.000, that the lands and air spaces around airport with the noise intensity level of more than 75dB measured with WECPNL are not recommended for settlement, schools, and hospitals. There is sufficient scientific evidence that the noise has influences on ischemic heart disease, hypertension, hearing impairment, sleep disturbance, duodenum and gastric disorders.<sup>3</sup> In line with that evidence, Hartono<sup>4</sup> also reported that there was an increase in the dyspepsia syndrome episode towards the workers working at the textile factory of PT. Kusuma Hadi Santosa in Karanganyar. In the meantime, the number of research on the correlation between the noise and human immune system is still limited.<sup>5</sup>

One of the noise impacts is stress. The stress brings about different reactions along the hypothalamic – pituitary– adrenal (HPA) axis; among them are the increase of adrenocorticotrophic hormone (ACTH) and corticosteroid.<sup>6</sup> Many researches have been conducted by experts to find out the correlation between the stress and the human immune system. As a result, there is a new medical field called psychoneuroimmunology. In relation to this breakthrough in the medical field, the scientists intend to find out the mechanism of psychological factors affect to the human immune system.<sup>7</sup> According to the psychoneuroimmunology perspectives, the immune system is very influenced by the hormonal system performance from the HPA axis and the sympathetic-adrenal medullary (SAM) axis.<sup>8</sup>

The data of several researches using animals as their objects of experiments prove that the stress inhibit the trafficking of neutrophils, macrophages, NK cells, and T lymphocytes or B lymphocytes. The experiments on the animal also show that the stress is proven to inhibit the production of chemokines, proinflammatory cytokines so that it

results in adaptive immune responses and impair effector functions of macrophage, lymphocytes, and NK cells.<sup>8,9</sup>

Natural Killer cells is an important components of the innate immune system, owing to their cytokine production and cytolytic activity against target cells. Human NK cells comprise  $\pm 15\%$  of all lymphocytes.<sup>10</sup> Since the existence of the NK cells is known without the presence of any stimulus from the host and is not required in the long activation period, there has been a hypothesis that the NK cells are truly prepared for frontline prevention mechanism towards the development of tumor cells and virus cells.<sup>11</sup> The NK cells are also one of the leukocyte subsets, which are responsive to the physiological and psychological stress.<sup>12</sup>

The activity of NK cells can be measured by using <sup>51</sup>Cr release cytotoxicity assay. In this measurement, the target cells are labeled by using <sup>51</sup>Cr and are mixed with effector cells with varied ratio. The target cells are lysed by effector cells, and intracellular Cr is released. After 4 hours at 37°C, the radiation of <sup>51</sup>Cr release is measured and expressed in percent specific lysis.<sup>13</sup>

The activity of NK cells (cytotoxicity) can also be measured by using non-radioactive method, a modification of procedure conducted by Andalib *et al.*<sup>14</sup> The measurement is based on the ability to lyse the target cells (tumor cells K562). The number of target cells is analyzed by using flowcytometric.

Based on the above elaborations, this study is aimed to find out the difference of NK cell activities of the people live in the aircraft noise area of Adi Sumarmo Airport Boyolali, Surakarta, Indonesia.

## **MATERIALS AND METHODS**

The materials employed in the research among others were venoject heparin tube, micro-tube of 0.5 mL, microtube of 1.5 mL, 1 set of micropipet, and 2 boxes of disposal gloves. For separating peripheral blood mononuclear cells (PBMC), 4 bottles of Ficoll Histopaque of 100 mL each was used. For working solution, RPMI 1640 + 10% FCS (Gibco, Germany), supplemented with L-Glutamine, 100 µg/mL streptomycine, 100 µg/mL penicillin adding 0.5 µg/mL Propidium Iodie (PI, Sigma). For the target cells, ATCC-K562 cell line was used. The

apparatuses used to measure the noise level were Sound Level Meter (SLM) with the trade of Extech, Model 407735 made in Japan. To measure the activity of NK cells, Flowcytometer FACS (Becton-Dickinson, USA) was used.

This research is an observational analytic with Cross Sectional design. It was conducted to the inhabitants around the runway of Adi Sumarmo International Airport, particularly in Dibal village and in Gagak Sipat village, Ngemplak Sub-district, Boyolali District. The sample population was all of the inhabitants of Dibal village and Gagak Sipat village, Ngemplak sub-district, Boyolali regency who fulfilled the following criteria. The inclusive criteria were female, married, housewife, aged 20-40 years old, living in the area for at least 1 year. Exclusive criteria were consuming non-herbal or herbal medicines, being pregnant, suffering from ear disease/deft, suffering from infectious disease (influenza/commond cold or diarrhea) and suffering diabetes mellitus.

The respondents who fulfilled the criteria were selected by means of simple random sampling. The sample size was calculated using the formula of Snedecor and Cochran or by using Win Episcopo 2.0 with estimated difference between means ( $\alpha = 0.05$ ). For an expected confidence level of 95%, the expected significance level was 95%. Based on the results of previous studies in the area, the number of lymphocytes  $S_D$  was 700;  $m_1$  was  $3.6 \times 10^3/\mu\text{L}$ ;  $m_2$  was  $2.5 \times 10^3/\mu\text{L}$ , and the number of subjects per group was 13.<sup>9</sup> The total number of subjects was 39, who were divided into 3 groups, on the basis of the distance of their residential area from the runway. Group 1: respondents whose living area is less than 500 meters from the tip of the runway with the noise intensity of 92.29 dB measured with the scale of WECPNL (noise-exposed Group 1). Group 2: respondents whose living area is between 500 and 1000 meters from the tip of the runway with the noise intensity of 71.79 dB measured with the scale of WECPNL (noise-exposed Group 2). Group 3: respondents whose living area is greater than 1000 meters from the tip of the runway with the noise intensity of 52.17 dB measured with the scale of WECPNL (noise-exposed Group 3).<sup>15</sup>

The noise measurement was performed through two ways: namely: 1) the noise was

measured when the aircrafts passed over the areas, and 2) the surrounding environment's background noise without the influence of the aircraft noise. The noise was measured by using Sound Level Meter (SLM).

In each area of the research, the measurement was conducted at three different points with portable LSM, and the acoustic physical parameter was measured in dB with A load. The LSM was placed with its filter parallel to the ears. The SLM was set up at its maximum function of value to measure the peak noise level when the aircrafts passed over the areas so that the background noise level could be blocked. The acoustic physical parameter was recorded based on the peak noise level occurring in the areas when the aircrafts were going to take off and land on, and the hours when such a noise level occurred were also recorded. The noise level was rated by using WECPNL. Its equation was as follows:

$$\text{WECPNL} = \text{dB(A)} + 10 \log N - 27$$

$N = N_1 + 3N_2 + 10N_3$ ; dB(A): the average decibel score of each peak level of aircraft activity in a day; N: The number of arrival and departure of aircrafts in 24 hours;  $N_1$ : The number of arrival and departure of aircrafts between 07.00 and 19.00 Western Indonesia Time;  $N_2$ : The number of arrival and departure of aircrafts between 19.00 and 22.00 Western Indonesia Time;  $N_3$ : The number of arrival and departure of aircrafts between 22.00 and 07.00 Western Indonesia Time.

The result of measurement in the scale of dB(A) was then converted into WECPNL in accordance with the number of aircrafts passing over the areas for 24 hours. The score of WECPNL was obtained from the average score of maximum dB(A) in a day and the number of aircrafts that passed over the areas in certain hours, which was included in N. The physical parameter of the background noise was obtained through the data taken within 10 minutes of each hour in which the data were taken in every 5 second, and distributed evenly. The measurement was performed during the operating hours of the airport from 06.00 to 19.00 Western Indonesia Time.<sup>15</sup>

## Subjects

Before the samples of the research were specified, the subject criteria-related data and the characteristics of its respondent (age, sex type, occupation, etc) had been documented through questionnaire. The respondents who fulfilled the criteria were taken through a simple random sampling technique. The total number of respondents was 39, and were divided into 3 groups

The activity or cytotoxicity of NK cells was measured by using a non-radioactive method, which was a modification of procedure conducted by Andalib *et al.*<sup>14</sup> Peripheral blood of 10 mL was taken by using syringe, and placed in the venoject tube which contained saturated solution of Heparin. The lymphocytes were separated by using Ficoll-Hypaque gradient technique. Lymphocytes were isolated, washed, and brought to a concentration of  $5 \times 10^5$  cells/mL in RPMI 1640+10% FCS (Gibco, Germany).

Target cell (K562 tumor cell line) maintained in continuous suspension culture in RPMI 1640+10% of FCS, supplement with L-glutamine, 100 µg/mL streptomycine and 100 U/mL penicillin. A working solution was prepared by adding 0.5 µg/mL propidium iodide (PI, Sigma) in RPMI 1640+10% FCS. The lymphocytes (as effectors) and K562 (as target) cell lines were mixed and cultured in the same tube with effector. Target ratio of 50:1 respectively. Briefly, the tubes containing the mixed cells were centrifuged for 7 minutes at

250 x g at room temperature, then kept in 37°C for 10 minutes in the water bath, then the tubes were resuspended.

In the working solution, a concentration of  $1 \times 10^5$  cells/mL was prepared to avoid recycling of NK cells. The samples were then incubated for 1.5 hours in the incubator at 37°C, 5% of CO<sub>2</sub>, then the cells concentration was brought to  $1 \times 10^6$  cells/mL and was ready for running by flowcytometry. The result of the measurement was read twice by two different observers. In order to monitor the spontaneous death rate, the only target cells (without effector cells) were incubated accompanied with the processing. The final concentration of  $1 \times 10^5$  cells/mL was used as control. The cells were analyzed with a FACScalibur flow cytometry. The activity of NK cells was calculated based on the percentage of dead target cells (K562) in the tube which contained effector cells, subtracted by the percentage of dead target cells in the control tube (without effector cells), divided by the total number of cells (100%), and subtracted by the dead target cells in the control tube (without effector cells).

The study has been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University, Yogyakarta

## RESULTS

The data of noise intensity level are shown according to the scale of WECPNL and the activity of NK cells of each group is presented in TABLE 1.

TABLE 1. The result of noise intensity level measured with WECPNL and the activity of NK cells of each group

No.	Group 1	Group 2	Group 3	p-value
1. The intensity level (dB)	92.29	71.49	52.17	
2. The activity of NK cells (%)	$12.50 \pm 3.25^a$	$17.20 \pm 3.06^b$	$22.33 \pm 6.30^c$	0.000

**Notes:** The different letters a, b, and c at one row show that there is a real difference in the test with Anova, which is followed up with Post Hoc Test with  $\alpha = 0.05$ .

Based on TABLE 1, it can be seen that the intensity levels in area 1, 2, and 3 were 92.29dB (WECPNL), 71.49dB (WECPNL), and 52.17dB (WECPNL) respectively. From the result of measurement, it can be seen that the three areas

had different real intensity levels. TABLE 1, also shows that the activity of NK cells of the respondents of Groups 1, 2, and 3 were  $12.50 \pm 3.25\%$ ,  $17.20 \pm 3.06\%$ , and  $22.33 \pm 6.30\%$ , respectively, showing that there was a significant average difference of

the activity of NK cells among the three groups as shown by the value of  $p = 0.000$  ( $\alpha = 0.05$ ). The measurement was continued with Post Hoc Test showed there was a significant average difference of the activity of NK cells between Group 1 and Group 2, between Group 2 and Group 3, and between Group 1 and Group 3 as shown by the value of  $p < 0.05$ . The detailed information of the activity of NK cells of each group can be seen in FIGURE 1, 2, and 3.

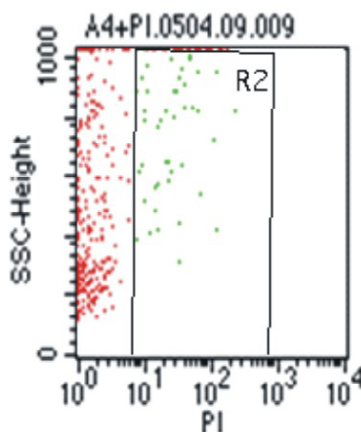


FIGURE 1. The comparison between the living target cells (K562) labeled in red color and the dead target cells (K562) labeled in green color of Group 1

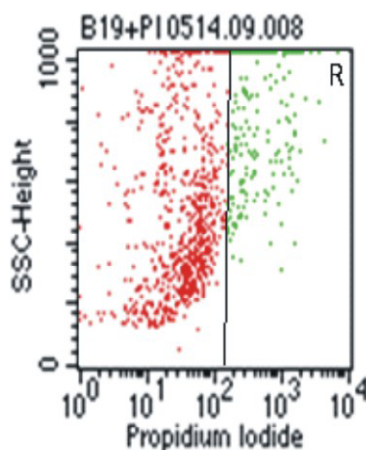


FIGURE 2. The comparison between the living target cells (K562) labeled in red color and the dead target cells (K562) labeled in green color of Group 2

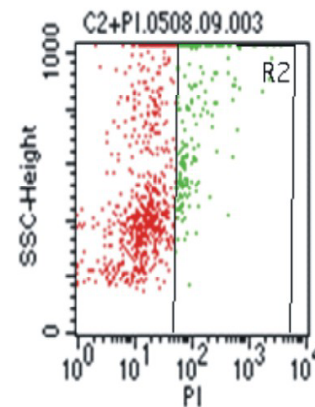


FIGURE 3. The comparison between the living target cells (K562) labeled in red color and the dead target cells (K562) labeled in green color of Group 3

## DISCUSSION

The results of this research showed that the respondents around the airport who were exposed to the aircraft noise with the intensity level of 92.29dB measured with the scale of WECPNL for more than one year suffered more from annoyance (stress), which lower the activity of NK cells than the control group exposed to aircraft noise with the intensity level of 52.17 dB measured with the scale of WECPNL. Meanwhile , the treatment group of respondents who were exposed to the aircraft noise with the intensity level of 71.49 dB measured with the scale of WECPNL also suffered more from annoyance which lower the activity of NK cells than the control group.

Those results were similar to the one reported by Andalib *et al.*<sup>14</sup> who conducted a research towards 45 women suffering from chronic stress due to recurrent spontaneous abortion. The result of this research showed that there was a high stress undergone by those suffering from the recurrent spontaneous abortion, and such a stress condition resulted in the decrease of the activity of NK cells. Similar results were also reported by Suzuki *et al.*<sup>12</sup>, Morikawa *et al.*<sup>13</sup>, and Nagao *et al.*<sup>16</sup>

The effects of noise on health depend on length of noise exposure, types of noise, and individual sensitivity. High intensity noise is more annoying than noise of low intensity, whilst intermittent noise is more annoying than continuous noise. Women are more sensitive in response to noise than men.

In the category of intermittent noise, aircraft noise is significantly more annoying. Chronic exposure to noise is thought to bring about significant impacts if it happens for more than a year.<sup>5,17</sup>

Several findings of researches with animal model showed that the increase of glucocorticoid and catecholamine due to the stress in a long term will affect the NK cells activity and the number of NK cells.<sup>8</sup> A number of cells in the immune system including NK cells have glucocorticoid and catecholamine receptors (epinephrine and norepinephrine). If a cell has glucocorticoid receptors, it will become a target cell. There are two types of receptors for glucocorticoid, namely mineralcorticoid receptors (MR) and glucocorticoid receptors (GR). Corticosteron has higher affinity towards MR than toward GR. As a result, in a low level of glucocorticoid of the blood, the glucocorticoid is more easily bound to MR. Meanwhile, the glucocorticoid will only be bound to GR if its level is high in the blood or tissue (i.e. in stress condition). In the immune cells, the receptors that plays the role is GR.<sup>8,18,19</sup>

It is believed that the function of glucocorticoid hormone directly inhibits the process of genetic transcription. In 1995 there were several publications presenting data that the glucocorticoid has opposing functions towards the activity of NF-kB (NF-kappa Beta). There are several cytokines produced by Th cells and macrophages whose operations are controlled by NF-kB. Therefore, if glucocorticoid can inhibit the activation of NF-kB, several cytokines can be deactivated. This will cause the decrease in the immune functions.<sup>8,18,19</sup>

In addition, it has been known that catecholamine also take the role in the immune system changes including cell proliferation, antibody and cytokine production, cytolytic activity, and cell movement. The operation of catecholamine is the same as that of HPA-axis. Along with the increase in glucocorticoid hormone produced by adrenal cortex, the activity of HPA-axis also increases the production of catecholamine by adrenal medullar. In the adrenal medullar, synthesis and secretion of epinephrine and norepinephrine take place. In human beings, 80% of the catecholamine produced by adrenal medullar are epinephrine. Norepinephrine is released by the sympathetic nervous.<sup>8,18,19</sup>

In the activity of chronic SAM (sympathetic-adrenal-medullary), epinephrine and norepinephrine can disrupt the immune functions. The course from the sympathetic nervous system to the immune system is based on the observation on the noradrenergic sympathetic nervous ends which comes from CNS and goes to two lymphoid tissues, both primary and secondary ones. It proves that the sympathetic nervous ends have relation to the immune cells. In such a relation, the sympathetic nervous ends release norepinephrine. Furthermore, when the epinephrine is released by adrenal medullar into the blood circulation, it is bound to the immune cells by adrenergic receptors, and this produces the same effects as the sympathetic nerves when they are directly stimulated. The catecholamine has effect on the target cells through adrenergic receptors and several immune cells such as lymphocytes, NK cells, and macrophages, which express adrenergic receptors.<sup>8,18,19</sup>

The second theory is based on the concept of Selye, that is, general adaptation syndroma (GAS). Selye suggests the impacts of stress on the immune system including the activity of NK cells which are divided into three stages, namely: alarm stage (activation), adaptation stage, and exhaustion stage.<sup>7</sup>

It is known that the continuous recurrent noise will bring about stress. Stress (psychologically and physiologically) can activate hypothalamus, and the activated hypothalamus will increase the efferent activity of nervus vagus and stimulate anterior hypophysis.<sup>20,21</sup> If the stressor exposes everyday with certain intensities and frequencies as well as certain durations, it will bring about such stress conditions as alarm stage (activation), adaptation stage, or exhaustion stage. In the alarm stage, the noise exposure will be responded as a stressor and caught by PVN (Paraventricular Releasing Factor) and cells at locus cereleus noradrenergic center in hypothalamus. The two cells will be activated or undergo the stress mode of Stage 1 and then secrete ACTH. The pituitary cells undergo the stress mode of Stage 1 or activation. Then, ACTH is caught by cells, and the cells at the cortex of the adrenal glands secrete glucocorticoid, furthermore, the cells at the medulla of adrenal glands secrete epinephrine and (EPI)-norepinephrine (NE). Both of the cells at the cortex and medulla of the adrenal glands

undergo the stress of mode Stage 1 or activation stage. Because all of the sub-population of leukocytes including NK cells has receptor for the cortisol (Receptor of Glucocorticoid), the cortisol can modulate the activity of NK cells.<sup>8</sup>

The changing towards gene expression mediated by hormone glucocorticoid and catecholamine can modulate the immune functions.<sup>8</sup> In the normal condition, the immunocompetent cells are in the homeostatic condition. In the alarm stage, the stressor will be responded with the increase of high cortisol levels, and followed by the increase number of NK cells (CD56<sup>+</sup>CD16<sup>+</sup>CD3<sup>-</sup>) and the increase activity of NK cells. If the stressor is increased, the adaptation stage will occur as shown by the decreasing cortisol level, but the decrease is still above the normal level. Furthermore, if the intensity, frequency, and length of exposure of the stressor are increased, the exhaustion stage will be present or the immune functions decrease as indicated by, one of them, the decrease of the activity of NK cells. In the exhaustion stage, the cortisol level is a little bit above the normal level, but lasts for long period of time.<sup>7,8</sup>

In Group 2, the respondents were exposed to aircraft noise with the intensity level of 71.49 dB measured with the scale of WECPNL for more than one year, probably they have already underwent the stress mode of exhaustion stage. It was indicated by the significant difference of the activity of NK cells between Group 2 and Group 3 (control groups). The higher of intensity level to 92.29 dB (Group 1) would be lower activity of NK cells as shown by the difference of the activity of NK cells in Group 1 compared to Group 2 or Group 3.

## CONCLUSION

Based on the analysis of the data, a conclusion is drawn that the aircraft noise at the intensity level of 71.49 dB measured with WECPNL with the length of exposure of more than one year can cause a stress at the exhaustion stage towards the inhabitants around Adi Sumarmo International Airport, Boyolali, Surakarta, Indonesia as shown by the low of NK cells activities. The higher intensity level to 92.29 dB will be lower the activity of NK cells.

Based on the results of the research, preventive measures are needed to deal with the aircraft noise so that it will not be producing more bad impacts towards the inhabitants around Adi Sumarmo International Airport, Boyolali, Surakarta, Indonesia. One of the efforts that should be considered is to relocate the inhabitants to safer settlements.

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